REMARKS

Status of Claims

This paper amends claims 1, 3-4, 9, 30, 34-35, 37, and 43-44, cancels claims 2, 14, 33, and 42, and adds claims 57-61. Support for the amendment to claims 1, 9, and 30, and new claim 57 is found generally throughout the Specification and specifically at [0009]. Support for new claims 58-61 is found, for example, at [0074]. Claims 9 and 37 have been amended to more clearly define Applicant's claimed invention. Claims 3-4, 34-35, and 43-44 have been amended to correct the dependency in view of other claim cancellations. Claim 44 has also been amended to replace "LC" with "liquid chromatography." No new matter is added by these amendments. After the amendments set forth herein are entered, claims 1, 3-4, 6-13, 15-17, 30-32, 34-41, and 43-61 are pending and under examination.

Claim Objections

The Examiner finds claims 2 ,14, 33, and 42 objectionable as being substantial duplicates of the claims from which they depend. The objectionable claims are canceled herewith. This objection may be withdrawn.

Rejection under 35 U.S.C. § 112, Second Paragraph

Claims 1-4, 6-17, and 30-56

Claims 1-4, 6-17, and 30-56 stand rejected as allegedly being indefinite. Specifically, the Examiner questions whether the claimed sample contains either or both of unlabeled and labeled organic acids. The Examiner interprets the claims as covering the "conventional usage of a labeled internal standard for quantifying an analyte of a similar nature by mass spectrometry." Office Action at page 2.

For clarity, Applicant notes that the claims do not require *a priori* knowledge of whether the sample contains an unlabeled organic acid; hence the recitation in step (a) that the sample is

"a sample suspected of containing the unlabeled organic acid to be measured." It is only after the determination is performed that the practitioner can know with certainty whether the sample did or did not contain an unlabeled organic acid of interest. With respect to the Examiner's allegation that the claims encompass the "conventional usage of a labeled internal standard," Applicant notes that it is not clear what is meant by "conventional usage." However, Applicant agrees with the Examiner to the extent that the claims require the addition of a known amount of an oxygen-18-labeled organic acid, quantification of the amount of that labeled organic acid by mass spectrometry, and use of that quantified amount to infer the amount of unlabeled organic acid (if any) that is present in the sample. Accordingly, Applicant respectfully submits that the rejected claims are not indefinite and that this rejection should be withdrawn.

Claims 7-8, 12-13, 31-32, and 40-41

Claims 7-8, 12-13, 31-32, and 40-41 stand rejected as allegedly being indefinite. Specifically, the Examiner alleges that it is unclear what the terms "enrichment" and "chemical modification" refer to. Applicant respectfully traverses this rejection.

Applicant submits that it is clear from the Specification and common usage in the art that "enrichment" refers to the act of concentrating an analyte and "chemical modification" refers to subjecting a molecule to a chemical process such as derivatization. Specification at [0060]-[0061]. In particular, enrichment is used to improve the detectability of an analyte (e.g., an organic acid), including using GC-based methods. Specification at [0060]. "Chemical modification" includes derivatization for the purpose of improving the volatility of an organic acid in order to facilitate separation by gas chromatography ("GC"). Specification at [0061]. Common derivatizing techniques include (a) the preparation of methyl esters using BF3/methanol or diazomethane, (b) the preparation of trimethylsilyl derivatives, and (c) the preparation of methyl-(tert-butyldimethylsilyl)-derivatives. Specification at [0061]. Accordingly, Applicant submits that these terms are well-known by the skilled artisan and clearly defined and/or exemplified in the Specification. This rejection is traversed and should be withdrawn.

Rejections of claims under 35 U.S.C. § 103(a)

All examined claims stand rejected under 35 U.S.C. § 103(a) as allegedly obvious over Peterson et al. (J. Lipid Res., 29: 94-101, 1988; "Peterson") in view of Nguyen et al. (US 2005/0070023; "Nguyen") either alone or in further combination with Pang (HKMJ 2: 264-273, 1996). The Examiner reads Peterson as disclosing the synthesis and mass spectrometric (MS) detection of oxygen-18 labeled glycolic acid (a hydroxyl mono-acid). The Examiner acknowledges that Peterson does not disclose using this oxygen-18 labeled acid as an internal standard but turns to Nguyen, alleging that Nguyen discloses the use of organic acids labeled with stable isotopes as internal standards for MS analysis. Finally, the Examiner relies on Pang to demonstrate that prior art measured organic acid levels in urine to diagnose metabolic disorders. Applicant respectfully traverses this rejection.

Claims 1, 3-4, 6-13, 15-17, 30-32, 34-41, 43-56, and new claims 57-61

Independent claims 1, 9, 30, and 37 each require (or have been amended to require) that the sample under analysis is a biological sample. Specifically, claims 1 and 9 have been amended to affirmatively require the use of a biological sample. Claims 30 and 37 each encompass methods for diagnosing an individual by assessing a sample from the individual. Therefore, the sample required by claims 30 and 37 is necessarily a biological sample. It is the requirement for the use of biological samples that renders the claims non-obvious over the cited prior art.

The Examiner relies on Peterson as the only reference that actually synthesizes and detects by MS an oxygen-18 labeled organic acid. Peterson prepares [^{18}O]-glycolic acid by heating choloroacetic acid in the presence of [^{18}O]-H_2O. Next, Peterson derivatizes the [^{18}O]-glycolic acid using bis(trimethylsilyl) trifluoroacetamide (BSTFA), neutralizes the derivatized sample, and detects the derivatized [^{18}O]-glycolic acid by MS. Peterson et al. at pp. 95-96.

There is nothing in Peterson that teaches or suggests that [^{18}O]-glycolic acid is suitable for use as an internal standard with a biological sample. As noted above, Peterson creates [^{18}O]-glycolic acid with a substitution reaction in which one or more of the carboxylic acid and the alcoholic [^{16}O] atoms in glycolic acid is substituted with [^{18}O] from the solvent, [^{18}O]-H_2O. Peterson's [^{18}O]-glycolic acid is then analyzed in a sample devoid of any other oxygen-containing compounds, such as proteins or other organic acids, that may provide an opportunity to re-exchange the [^{18}O] for an [^{16}O]. Because generation of the [^{18}O]-glycolic acid occurs by a mere replacement reaction in the presence of [^{18}O]-H_2O, Peterson provides no reasonable expectation that the [^{18}O] atoms from the [^{18}O]-glycolic acid, once labeled, would not re-exchange with other carboxylic acid and/or alcoholic oxygen atoms in the plethora of unlabeled biological molecules (e.g., proteins) and/or other organic acids found in a complex biological samples such as urine.

Furthermore, Nguyen (relied upon by the Examiner) demonstrates that this potential problem was known in the art and teaches away from using an isotopic label such as [^{18}O] under circumstances when the labeled analyte is generated by a simple isotope exchange reaction such as that demonstrated by Peterson. In discussing the use of isotopic labels, Nguyen states:

> The ideal [internal standard], however, must not contain any labeled isotope that can be exchanged for the unlabeled isotope under particular sample preparation conditions.
>
> Nguyen et al. at [0005].

Nguyen goes on to characterize suitable stable isotopes as follows:

> Most often the synthesis of stable isotope internal standards is not simply an isotope exchange reaction. Easily exchangeable atoms are usually avoided due to possible re-exchange during sample preparation steps.
>
> Nguyen et al. at [0006]. Emphasis added.

Thus, the use of the [^{18}O]-glycolic acid of Peterson for an internal standard, as alleged by the Examiner, is exactly contrary to the admonitions of Nguyen. As discussed above, the [^{18}O]-

glycolic acid of Peterson is formed by a simple exchange reaction; exactly the type that could result in re-exchange during sample preparation. The Examiner has provided no reasoning as to why the skilled artisan would ignore Nguyen's express teaching away and have any reasonable expectation of success in the use of the [^{18}O]-labeled organic acids as internal standards.

The affirmative teachings of Nguyen do not remedy the deficiencies of Peterson, nor do they negate Nguyen's general teaching away from the use of free-substituted isotopic labels, which include [^{18}O] in the labeling reaction of Peterson. Although Nguyen generally discloses that internal standards may be isotopically labeled with any number of stable isotopes (Nguyen et al. at [0005]), Nguyen synthesizes a deuterium-labeled organic acid. Nguyen et al. at [0027]-[0032]. Thus, Nguyen does nothing to demonstrate that [^{18}O]-labeled organic acids may be used as internal standards for mass spectrometry.

Pang also fails to remedy the deficiency in the *prima facie* case of obviousness based on Peterson and Nguyen. Pang merely discloses that the levels of organic acids may be measured in urine as an indicator of metabolic disease. However, nothing in Pang is related to the MS detection of organic acids, let alone the use of [^{18}O]-labeled organic acids in biological samples.

In contrast to the simple system of Peterson, which contains only glycolic acid as a potential [^{18}O]-acceptor in a non-biological sample, and in contrast to Nguyen's admonitions, Applicant has demonstrated that [^{18}O]-labeled organic acids are stable in human urine and suitable for use as internal standards. Example 7 evaluates the stability in human urine during prolonged liquid-liquid extraction of multiple [^{18}O]-labeled organic acids, including hydroxyl mono-acids (2-hydroxy-butyric acid, 3-hydroxy-2-methyl butyric acid, 2-hydroxy isocaproic acid, and 4-hydroxy phenyl acetic acid), di-hydroxyl mono-acids (glyceric acid), glycine conjugates (gutyryl glycine and crotonyl glycine), di-acids (glutaric acid), and oxo acids (succinyacetone), that were labeled with 1, 2, 3, or 4 [^{18}O]-atoms. Specification at [0074]-[0083]. In this experiment, human urine was spiked with the labeled organic acid and subjected to liquid-liquid extraction. The organic acids were then derivatized and detected by GC-MS. For each organic acid, the proportion of each of the labeled forms and the unlabeled form stayed relatively

constant following an 80 minute liquid-liquid extraction prior to derivatization, and the proportion of unlabeled organic acid increased only slightly following a 120 minute extraction. These results prove that the isotopic label on the organic acids remained stable (i.e., did not rearrange with unlabeled H_2O or other urinary biomolecules) during a prolonged extraction procedure commonly used for processing biological samples.

Example 8 expands upon the results of Example 7. Here, urine samples were spiked with individual [^{18}O]-labeled and unlabeled organic acids and subjected to the same extraction, derivatization, and MS detection procedures as used in Example 7. Specification at [0084]-[0086]. As illustrated in Figure 11 and discussed at [0086], detection of the [^{18}O]-labeled organic acid maintained linearity over a concentration range of 10-600 nM (r^2=0.9991). These findings are significant because, in addition to the normal biomolecules found in urine, the [^{18}O]-labeled organic acid was used in the presence of the same unlabeled acid which simulates the conditions under which the [^{18}O]-labeled organic acids actually will be used.

Example 9 further increases the complexity of the biological sample in which the [^{18}O]-labeled organic acid was used. Specification at [0087]-[0095]. In this example, a variety of [^{18}O]-labeled organic acids were used as internal standards in urine samples spiked with a mixture of organic acids. The spiked samples containing the internal standards were subjected to the same extraction, derivatization, and detection procedures as outline above. In each case, the [^{18}O]-labeled organic acid remained stable and performed well as a calibration standard.

In sum, Peterson is the only prior art reference cited by the Examiner which detects an [^{18}O]-labeled organic acid by MS. Peterson does not detect an [^{18}O]-labeled organic acid in a biological sample, as presently claimed, nor does Peterson teach or suggest that an [^{18}O]-labeled organic acid may be suitable as an internal standard for this purpose. Peterson uses only very simple solutions in which the [^{18}O]-labeled organic acid is never in contact with any other potential [^{18}O]-acceptor, such as those that would be found in a biological sample. In contrast, Applicant has demonstrated that [^{18}O]-labeled organic acids are suitable for use as internal standards in biological samples and perform well as calibration standards even following

extensive liquid-liquid extraction procedures; a finding not taught or suggested by the prior art. Accordingly, the requirement that the claimed methods be applied to biological samples is not *prima facie* obvious. For this reason alone, this rejection is traversed and should be withdrawn.

Claims 58-61

Claims 58-61 have been newly added and require that the biological sample under assessment is acidic. This additional limitation provides a second independent basis for non-obviousness over the cited prior art. As discussed above, Peterson prepares [^{18}O]- glycolic acid by a substitution reaction with [^{18}O]-H_2O under acidic conditions in a solution that lacks other potential [^{18}O]-acceptors. Peterson also derivatizes the [^{18}O]- glycolic acid with BSTFA and neutralizes the solution with one equivalent of sodium hydroxide prior to detection. Peterson et al. at p. 95, right column.

In contrast to the Peterson method, claims 58-61 require that the biological sample in which the [^{18}O]-labeled organic acid is detected, is acidic. It is not obvious to use an acidic biological sample in view of Peterson. Because the initial isotope exchange reaction was initially conducted under acidic conditions, the skilled artisan would assume that the acidic conditions required by claims 58-61 would increase the probability for [^{18}O] rearrangement.

In contrast to the Peterson method and the expectation of the artisan, Applicant demonstrates that [^{18}O]-labeled organic acids perform well in acidic solutions. Specifically, in Examples 7-9, the pH of the urine samples was adjusted to 1 using 1N sulfuric acid. Specification at [0074].

Thus, the use of acidic biological samples is not obvious in view of Peterson's acidic isotope exchange reaction and subsequent neutralization prior to analysis. This provides a second independent basis for the non-obviousness of newly added claims 68-61.

Claims 9-13, 15-17, 37-41, 43-45, 59, and 61

Independent claims 9 and 37 require the use of a seven-way internal standard solution not taught or suggested in the prior art. Specifically, these claims require the use of at least one oxygen-18 labeled organic acid from each of the following organic acid classes: hydroxy mono-acid, dihydroxy mono-acid, dicarboxyl organic acid, hydroxyl dicarboxyl acid, tricarboxyl acid, glycine conjugate, and keto acid. The Examiner has failed to fully examine these claims, cite to any prior art, or provide any reasoning as to why these claims are obvious. Furthermore, in view of the teachings of Nguyen (cited by the Examiner), the artisan is discouraged from using, as internal standards, multiple organic acids with freely-exchangeable isotopic labels such as [^{18}O]. Thus, the requirement for multiple oxygen-18 labeled internal standards renders independent claims 9 and 37, and their dependent claims, non-obviousness.

CONCLUSION

Applicant believes that the present application is now in condition for allowance. Prompt and favorable action on the application is respectfully requested.

The Examiner is invited to contact the undersigned by telephone if it is felt that a telephone interview would advance the prosecution of the present application.

The Commissioner is hereby authorized to charge any additional fees which may be required regarding this application under 37 C.F.R. §§ 1.16-1.17, or credit any overpayment, to Deposit Account No. 19-0741. Should no proper payment be enclosed herewith, as by the credit card payment instructions in EFS-Web being incorrect or absent, resulting in a rejected or incorrect credit card transaction, the Commissioner is authorized to charge the unpaid amount to Deposit Account No. 19-0741. If any extensions of time are needed for timely acceptance of

papers submitted herewith, Applicant hereby petitions for such extension under 37 C.F.R. §1.136 and authorizes payment of any such extensions fees to Deposit Account No. 19-0741.

Respectfully submitted,

Date 10/12/10

By _____

FOLEY & LARDNER LLP
Customer Number: 30542
Telephone: (858) 847-6722
Facsimile: (858) 792-6773

Barry S. Wilson, Reg. No. 39,431
Anthony C. Kuhlmann, Reg. No. 57,147
Attorneys for Applicant